

# Seasonal Variation in Soil Organic Carbon

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Long-term changes in total soil organic C usually occur gradually. These long-term trends might be obscured by smaller, rapid changes in soil C due to seasonal inputs of plant residues, roots, and exudates, or decomposition of such inputs. Yet there is little, if any, data describing the magnitude of seasonal changes in soil C. If seasonal fluctuations in soil C are substantial, then important implications exist for accurate comparison of soil C between sites, between treatments, and even in the same experimental unit over time. Thirty-nine consecutive monthly soil samples were taken from a field experiment planted every year with winter wheat (*Triticum aestivum* L.) in the Pacific Northwest, United States. The variation in soil organic C was 14 to 16% of the mean over the 39-mo period in the top 250 kg m<sup>-2</sup> equivalent mass (~0- to 20-cm depth). Two to eight percent could be identified as a regular seasonal pattern. The no-till management system had the greatest seasonal fluctuation, and the timing of the annual maximum was different from that of the tilled soil management treatments. In the shallower soil layer (~0–7 cm), total soil organic C varied 12 to 29% in which 4 to 13% could be attributed to a 12-mo seasonal pattern. Given the small magnitude of changes in soil C being measured and modeled in many agricultural and natural systems, soil samples taken at a single point in time are likely to encounter substantial but hidden measurement variability. The variability may be compounded by factors of the timing of sampling in relation to natural soil organic matter cycles and differences in the cycle due to treatment and weather. Sampling plans, which account for seasonal fluctuation and the different fluctuation patterns under different soil situations, will improve measurement accuracy.

**Abbreviations:** STL, seasonal decomposition of time series by LOESS.

Efforts to measure changes in soil C rely on soil samples that compare different ecosystems or management treatments at the same point in time or compare a single treatment at two points in time separated by many years or decades. Both methods require us to assume that the samples produce valid, representative estimates of soil C for the soil condition being measured. We, therefore, have to assume that small differences in timing (a week to several months) will not affect our comparison of two sites or treatments or affect measurement of change occurring over many years or decades.

It is commonly accepted that soil organic matter consists of a large pool of very slowly changing, protected, or recalcitrant organic matter, plus several smaller pools under more rapid flux (Fierer et al., 2009; Kiem and Kögel-Knabner, 2003). The pools under rapid flux are usually assumed to be much smaller than the slow-to-change background pool. On the other hand, it is believed that soil C, especially in the less protected pools, is responsive to temperature, soil moisture, and plant growth, all of which follow a seasonal pattern.

Soil C is known to be spatially variable. Researchers have recommended up to 100 subsamples per measurement to achieve an estimate within 10% at a 95%

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confidence level at the field scale (Post et al., 2001). Studies of spatial variability rarely consider the possibility of seasonal variation, other than to recommend sampling at the same time each year (Allen et al., 2010; Pringle et al., 2011).

A search in the literature for seasonal trends in soil C reveals a large number of studies measuring respiration and microbial factors but very few analyzing total organic C. The following studies where soil was sampled at different times of the year report substantial variation in total soil C. In three mixed oak forest sites in Ohio, two samples taken about a month apart differed in total C by 28, 40, and 52% (Boerner et al., 2005). In the Canadian prairie, four measurements taken in spring, summer, autumn, and winter varied 26% at one grassland site and 53% at the other (Dormaer et al., 1977). In Michigan, the difference between April and June samples was 23% in a wheat field and 18% under poplar trees (Stoyan et al., 2000). These studies indicate that seasonal fluctuation could be a significant factor, but other factors may also be involved, such as artifacts due to sampling technique, fluctuations in soil water content and/or soil bulk density, identifying the same position in the soil profile, and sample handling differences such as drying temperature or inclusion of fine residue and detritus particles.

Another dataset where multiple samples were taken over a season is from a study at two agricultural research sites in Saskatchewan, Canada (Campbell et al., 1999a; Campbell et al., 1999b). Soil samples were taken up to 11 times over two 5-mo growing seasons. The measured soil C sometimes varied by 5000 mg kg<sup>-1</sup> during the 5-mo period in soils that contained about 25,000 mg kg<sup>-1</sup> organic C. Among the many crop rotations they studied, soil organic C appears to have varied from about 4 to 20% during the 5-mo period. In some cases, there appears to be a gradual seasonal cycle, which is similar between treatments; however, in others the samples taken less than 30 d apart differed by more than 10%. The measured variability is not necessarily all due to actual seasonal variability in soil C content. Some of the variability would be due to other sources of experimental error such as sample processing and spatial variability.

In summary, long-term changes in total soil C tend to be small (Post and Kwon, 2000), but there is considerable measurement uncertainty (Ogle et al., 2003). If seasonal variation exists, this would create the potential for serious errors based on single point-in-time estimates. For example, in relation to the cycle of the soil C response to weather and plant growth, the timing of a measurement taken 10 yr ago might not match the timing of a current measurement, and so an increase or decrease caused by seasonal changes might be seen as a product of the 10-yr time period instead of part of an annual, seasonal variation. Also, ecosystems or soil treatments might have different seasonal trends, and this could cause misleading treatment comparisons depending on the timing of a single soil sample in relation to the seasonal cycle of each treatment.

In the present study, the research objective was to determine if soil C undergoes seasonal fluctuations that complicate accurate evaluations of C changes and differences. The investigation

included three soil management treatments to see if they have different seasonal trends.

## MATERIALS AND METHODS

Three tillage treatments were compared in plots planted every fall with winter wheat near Pendleton, OR on a Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll) containing 21% fine to very fine sand, 69% silt, and 10% clay. The 3.6- by 53-m plots were replicated in four randomized, complete blocks. Fertilizer, crop management, and crop rotation were identical. Fertilizer N at 100 kg ha<sup>-1</sup> and P at 20 kg ha<sup>-1</sup> were applied at planting time, which was mid-October. Average harvested grain yield was 3910 kg ha<sup>-1</sup>, leaving about 260 g residue C m<sup>-2</sup>. The three tillage treatments were (i) no-till, (ii) tillage to incorporate crop residues after harvest and before planting, and (iii) a novel treatment where surface residues were raked onto a tarp, the plots tilled the same as the above tillage treatment, and then the surface residues spread back onto the plot surface.

Plots were sampled midmonth in a transect, moving the sampling site 30 cm down a crop row each month so the samples would be spatially close. A different transect location was chosen each year. The three cores that were combined for each sample were taken from the crop row, from halfway between rows, and from between the first two cores to represent both row and inter-row soil. Cores were segmented into six 5-cm depth increments. The total core cross-sectional area was 51.17 cm<sup>2</sup> (4.66-cm diameter cores, three per sample). Samples were weighed and spread to dry in an oven at 40°C within an hour of collection. Dry mass was determined for each sample and bulk density calculated using the core cross-sectional area and increment length. Analyses included water content (g g<sup>-1</sup> dry soil) and total C by dry combustion of the <1-mm soil fraction. The soil at the test site has no carbonates near the surface; therefore, total C is a measure of organic C.

To remove variation in soil bulk density as a factor in effective sampling depth and amount of soil contained in a sample, the C data was converted from depth increments in cm below the soil surface to equivalent soil mass per unit area (Wuest, 2009). Cumulative mass of organic C with depth was calculated for the depth increments of each sample and the equivalent mass-depths of 100 kg soil m<sup>-2</sup> and 250 kg soil m<sup>-2</sup> calculated by interpolation. A mass depth of 100 kg m<sup>-2</sup> is approximately 0 to 7 cm and 250 kg m<sup>-2</sup> is approximately 0 to 20 cm.

## Statistics

Seasonal decomposition of time series by LOESS (STL) (Cleveland et al., 1990), as presented by Cleveland for R (R Development Core Team, 2012), was used to search for a seasonal component among the monthly means of each treatment. This method searches for a periodic (here 12 mo) seasonal component by averaging data for each month over years (for other examples of this method, see Li et al., 2003; Randerson et al., 1999). These monthly averages are subtracted from the data. An

overall trend in the resulting data is determined by smoothing using locally weighted polynomial regression. Outliers are identified, their weighting factor reduced, and the process repeated. When the seasonal and long-term trend components converge on a stable solution, they are subtracted from the original data to produce the remaining, unexplained amount for each point. The final output consists of four plots: the original data, the seasonal component, the long-term trend, and the remainder.

The STL procedure is performed on individual treatments, so it does not test whether apparent differences between the soil management treatments are statistically significant. To test whether the treatments produced significant differences in soil C averaged over each of the three crop years (November–October), a generalized linear mixed model (Littell et al., 2006) was used. In addition, a generalized linear mixed model analysis of each of the 39 individual sample dates was performed to look for the treatment effect that would have been detected from single-point-in-time samples.

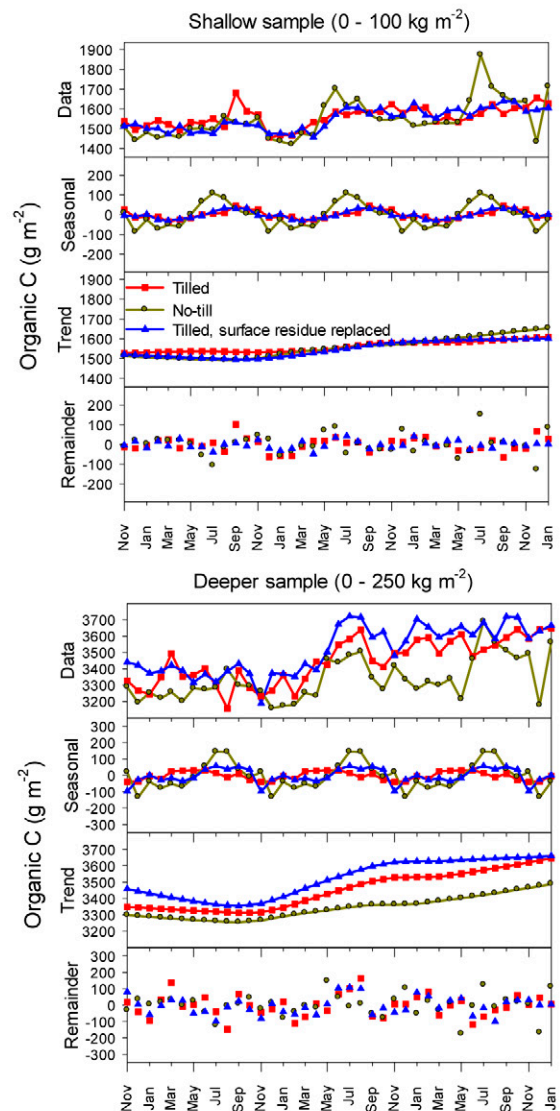
## RESULTS

A seasonal trend in soil organic C was evident for both sample depths. In the top 0 to 250 kg m<sup>-2</sup> (Fig. 1), the seasonal variation in total organic C represented 2 to 8% of the mean C level (Table 1). Over the 39-mo sampling period, total sample variation represented 14 to 16% of the mean soil C. In shallower samples (Table 1), the variation in soil C within treatments over time was an even greater proportion of average total C compared to the deeper depth.

For reference, temperature, precipitation (Fig. 2), soil bulk density, and soil water content (Fig. 3) are shown. The weather trend and soil C data appear to be related based on visual comparison of the fluctuations within the plots, but the three soil treatments do not respond to weather in the same way. As would be expected, soil bulk density changes also appear to be influenced by weather, although the year-to-year seasonal effects vary widely.

Treatments differed only in the degree of surface tillage and surface residue, but they created different seasonal patterns of soil organic C. The seasonal component of organic C for the no-till treatment measured in the 0- to 100-kg m<sup>-2</sup> depth (Fig. 1) had greater maximums and minimums than the two tilled treatments. It also had peaks that are a month or two earlier than the tilled soil treatments. The overall trend line indicates very similar soil C between the three treatments at the shallow depth. When analyzing a depth of 0 to 250 kg m<sup>-2</sup> (Fig. 1), the no-till treatment again demonstrated greater maximum and minimum seasonal fluctuations, but the overall trend for no-till soil C is substantially lower than the tilled treatments.

The tests of main effects of tillage treatment and crop year are shown in Table 2. This statistical analysis supports the trends detected by STL. For example, treatment effects on organic C are not significant for the shallow sample but are highly significant for the deeper sample, where Fig. 1



**Fig. 1.** Seasonal decomposition of time series by LOESS (STL) for soil organic C measured for 39 consecutive months. Two depth increments were analyzed, the top 100 kg m<sup>-2</sup> (approximately 0- to 7-cm depth) and the top 250 kg m<sup>-2</sup> (approximately 0- to 20-cm depth). The plots show the original data, with three graphs below it where the data has been partitioned into the seasonal component, the long-term trend, and any unexplained, remaining value. Within each set of four graphs the y-axes have equal range and tick spacing. The replicated plots were planted annually with winter wheat under three soil management treatments: (i) no tillage, (ii) tilled after harvest to 15 cm, incorporating surface residues into the tilled layer, and (iii) tilled as above but surface residues raked aside and replaced on the soil surface after the soil was tilled.

**Table 1.** Data range and seasonal component range as percent of the mean total organic C.

Treatment	0–100 kg m <sup>-2</sup> depth			0–250 kg m <sup>-2</sup> depth		
	Mean	Range	Seasonal range	Mean	Range	Seasonal range
All data	g m <sup>-2</sup>	%	%	g m <sup>-2</sup>	%	%
Tilled	1552	29	13	3431	16	8
No-till	1559	14	5	3444	14	2
Tilled, surface residue replaced	1550	29	13	3336	16	8
Tilled, surface residue replaced	1547	12	4	3513	15	4



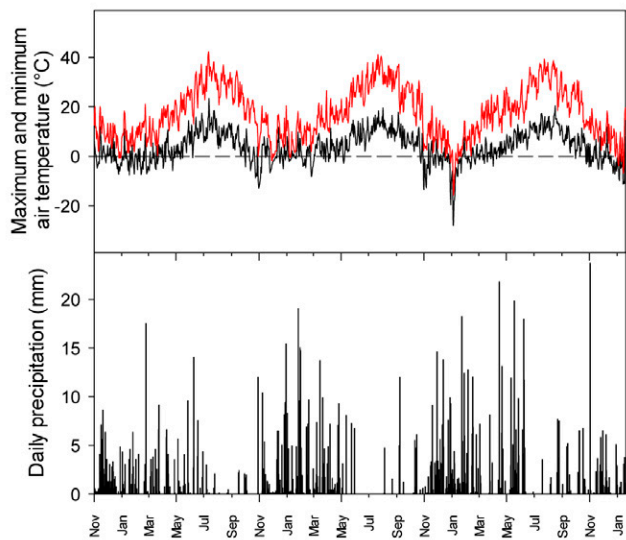


Fig. 2. Maximum and minimum air temperatures and daily precipitation for the sampling period.

shows a larger separation between treatment trends. An overall increase in organic C was significant over the 3-yr period.

The linear estimates for treatment by crop-year interactions shown in Table 2 are based on the means of four replications over 12-mo periods. If the data is instead analyzed as 39 individual samples, the organic C data for the 0 to 250 kg m<sup>-2</sup> depth resulted in only five sample dates with significant ( $p < 0.05$ ) differences between treatments (August of Crop Year 1, October of Crop Year 2, and January, April, and May of Crop Year 3). August of Crop Year 1 was one of only two samples where the tilled treatment gave the lowest soil C measurement (Fig. 1, deeper sample).

An indication of the spatial variability in soil C in this experiment can be derived from the variability between blocks within treatments on each sample date. A measure of this is the standard error of the mean (calculated by generalized linear mixed model,

Table 2. Generalized linear model Type-3 test of fixed effects for 36 of the 39 mo of organic C data divided into three 12-mo crop years. Month within crop year and replication (four blocks) were designated as random effects. Units of the least square estimates are g m<sup>-2</sup>. Estimates followed by different letters are significantly different at  $p < 0.05$ , with the experiment-wide error rate protected at  $p < 0.05$  by the simulate method (Littell et al., 2006). Treatment codes: T = tilled, N = no-till, R = tilled with surface residues removed and replaced after tillage. These means can be compared to 12-mo segments of the appropriate trend graphs in Fig. 1.

0–100 kg m <sup>-2</sup> depth			0–250 kg m <sup>-2</sup> depth		
Effect	p > F		Effect	p > F	
Treatment	0.5684		Treatment	<0.0001	
Crop year	0.0007		Crop year	<0.0001	
Trt × Cyrt	0.0414		Trt × Cyrt	0.0691	
Trt	Cyr	Estimate	Trt	Cyr	Estimate
N	3	1605 <sup>A</sup>	R	3	3632 <sup>A</sup>
R	3	1594 <sup>BA</sup>	T	3	3550 <sup>BA</sup>
T	3	1582 <sup>BA</sup>	R	2	3494 <sup>BC</sup>
T	1	1542 <sup>BAC</sup>	T	2	3410 <sup>DC</sup>
N	2	1541 <sup>BAC</sup>	N	3	3407 <sup>DCE</sup>
T	2	1536 <sup>BAC</sup>	R	1	3385 <sup>DCE</sup>
R	2	1531 <sup>BC</sup>	T	1	3327 <sup>DFE</sup>
R	1	1504 <sup>C</sup>	N	2	3313 <sup>FE</sup>
N	1	1493 <sup>C</sup>	N	1	3270 <sup>F</sup>

<sup>F</sup> Trt, treatment; Cyr, crop year.

$n = 4$ ) for treatment effects. Standard errors ranged from 46.5 to 121.0 g m<sup>-2</sup> over the 39 sample dates for the 0- to 250 kg m<sup>-2</sup> sample depth. Standard errors ranged from 29.7 to 112.2 g m<sup>-2</sup> for the 0 to 100 kg m<sup>-2</sup> sample depth. These standard errors are for samples composed of three cores, combined before weighing and analysis.

## DISCUSSION

Thirty-nine consecutive monthly soil samples demonstrated temporal variability in soil organic C. A substantial amount of the variation corresponded to a seasonal annual cycle. Some of the points in the remainder components of Fig. 1 appear to be serially (temporally) correlated. This remaining variability looks like it should be part of the seasonal component, but it was not partitioned into the seasonal component because the seasonal component only picks up variability that is consistent for a particular month over the entire measurement period. Soil C would be expected to respond to biologically-based measures such as a combination of soil water potential and degree days. Year-to-year differences in the timing of weather will push soil C reactions to earlier or later calendar dates (Brooks et al., 2011).

There are at least two important ramifications of this lack of synchrony between

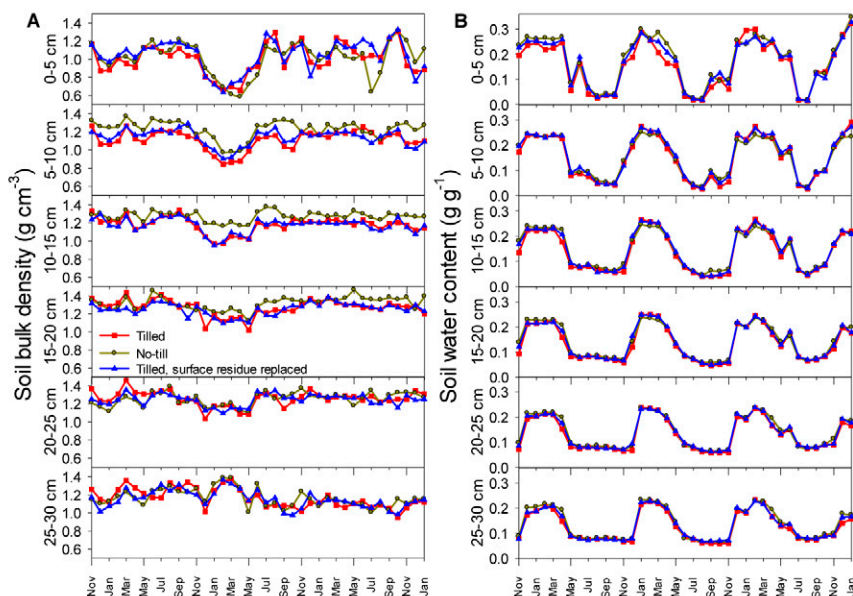


Fig. 3. Soil bulk density (a) and water content (b) for the 39 monthly samples.

the calendar and soil biology. First, the calendar-based seasonal component determined by STL is a conservative estimate of the range of soil C likely to be experienced in these soil management systems over the course of a year. Second, simply choosing the same calendar date for making soil measurements in a particular soil management system will not guarantee a reduction in the seasonal variability component.

Each of the three soil treatments were analyzed separately, and when superimposed on the same graphs (Fig. 1) they demonstrate generally (within a month or two) similar responses in shape and timing. If the treatments had involved crops with different growing seasons, they likely would have generated a greater contrast in seasonal patterns because of differences in timing of plant development, water use, litter quality, and other factors. Studies comparing multiple-year crop rotations would probably have seasonal and annual cycles of soil C extending the length of the rotation.

This research did not investigate the cause of differences among the treatments, but it is interesting to observe how the two tilled treatments were very similar in their seasonal cycle in the shallow sample but diverge in timing when the deeper sample is analyzed (Fig. 1).

There are several possible sources of seasonal variation in soil C. One is the short-lived C pool, generally considered to include particulate organic matter, microbial biomass, and living roots and root exudates. Live and recently living root mass in addition to root exudates are a largely unmeasured pool of organic C. Estimates of the contribution of roots to soil C are difficult to verify, but it is probable that roots play a larger role in total soil C than commonly acknowledged (Allmaras et al., 2004; Wuest and Gollany, 2013). There is difference of opinion whether the buildup of fine particles of plant residue or roots should be included in soil C budgets when much or all of it will decompose rapidly (Janzen, 2005; Gollany et al., 2013). Many researchers separate this particulate organic matter from mineral soil particles by density and report it separately. It is often still included as a component of total soil C, and in some soils this fraction represents the only statistically significant difference between soil management treatments, even over a period as long as 40 yr (Mikha et al., 2013).

In the present study, particulate matter small enough to pass through the sieve was not removed from the samples, and the light fraction and particulates inside aggregates were not measured separately. This means that the data presented includes fine residue particles, detritus, root fragments, and other free and aggregate-bound sources of recent organic addition. A significant portion of the seasonal variation in soil C would be due to these recently added organic pools. When particulate organic matter and microbial biomass were measured by Campbell et al. (1999a; 1999b) at two sites in Saskatchewan, Canada, the two fractions could explain about half of the variation in total soil C.

Other sources of variation in soil C measurements are sampling artifacts and spatial variability. Soil bulk density and measurement of soil bulk density vary with soil moisture (Hopkins

et al., 2009), soil freezing, tillage, seeding, and other operations. This can have a direct effect on quantitative and qualitative measurements that use the elevation of the soil surface as a primary datum. In studies where single depth increments are sampled using normal techniques, the samples cannot be corrected to equivalent mass, and variability in bulk density will add to variation in effective sample depth and soil C quantification. In the present research, a known surface area was sampled in 5-cm increments, and the dry mass of each sample was measured, thus, allowing interpolation to equivalent soil mass. This prevents surface bulk density variation over time or between treatments from causing unaccounted variation in effective sampling depth (Ellert and Bettany, 1995; Wuest, 2009). In hindsight, the author recommends that researchers sample deep enough so that equivalent mass approximating at least 30 cm can be calculated (even deeper is better). If intact cores are collected and dried, the desired equivalent soil mass can be weighed from the top of each core in the lab so that only one chemical analysis per depth increment is needed and interpolation is not required.

Janzen (2005) lists several problems that still need to be resolved to produce consistent, logical results in studying soil C. These include sampling to an adequate depth, treatment effects on soil bulk density causing different effective sampling depths, spatial variability within the treatment site, and inconsistent amounts of fresh crop residues becoming included in the samples. Sampling plans that address seasonal variability need to be added to this list.

## CONCLUSIONS

1. Soil organic C can undergo substantial fluctuations over the course of a year in seasonal patterns that differ with soil management. These fluctuations can be of a large enough magnitude to unwittingly obscure accurate estimation of C sequestration over years or decades.
2. Accurate determinations of organic C changes over time are going to require either an understanding of seasonal fluctuation patterns for a particular ecosystem or sampling plans that adequately average out seasonal variation. Comparisons between ecosystems or treatments will require assurance that the timing of samples does not bias the measurement to one treatment.
3. It is possible that seasonal variation can be reduced by processing soil samples to remove the light fraction, particulate organic matter, or some other component of soil organic matter, but this will still require that the remaining seasonal component be evaluated and also require a determination whether the removed fractions are or are not important components of the C sequestration being evaluated.

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